THE OXIDATION BEHAVIOR OF HYDROXYCINNAMATES OF WHITE WINES PRODUCED FROM EUROPEAN AND INDIGENOUS GRAPE VARIETES

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Abstract: The browning processes of experimental wines produced from indigenous grape varieties *Legenda*, *Viorica* and European grapes *Chardonnay*, *Sauvignon* have been studied. The browning processes are corelated with oxidation of the most important group of phenolic compounds of white wines – *hydroxycinnamates* (hydroxycinnamic acids and their tartaric esters, HCA). The degree of alteration of wine colour have been appreciated by using POM-test. The comparative antioxidant capacity of wines have been determined using method of kinetics competition of Crocin oxidative fading (Crocin Bleaching Assay) through peroxy radicals generated by 2,2'-Azo-bis 2-amidinopropan-diidroclorid (AAPH).

Key words: white wines, hydroxycinnamates (HCA), oxidation, spectrophotometry, POM-test, Crocin Bleachig Assay

Introduction

Moldovan wines are made from international and indigenous grape varieties. In recent years, Republic of Moldova has made a big leap in the growing of local grapes: *Feteasca Alba*, *Feteasca Regala*, *Feteasca Neagra*, *Rara Neagra*, *Viorica* and *Legenda* (*VitisVinifera L.*). *Viorica* and *Legenda* are used by Moldovan wineries for the production of quality white wines.

In order to best exploit the potential of local wines it is necessary to study the physico-chemical and organoleptic properties of them, polyphenol metabolism during processing of grapes, winemaking and storage of wine.

The importance of cinnamic compounds is well known [1]: these compounds are responsable for oxidative browning, process catalysed by polyphenol oxidase (PFO) or by ions and transition metals (preponderant Fe and Cu). The HCA determine largely the colour of white wines, the antioxidant properties and some aromas after the alcoholic fermentation. In this conext, it is important to evaluate the phenolic profile and antioxidant capacity of the wine, the oxidation behavior of phenolic compounds.

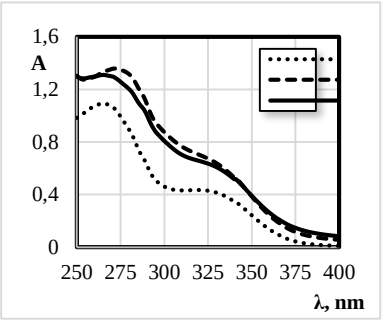
Materials and methods

The wines produced from 2 European grape varieties *Chardonnay* (Ch), *Sauvignon Blanc* (S) and 2 local grape varieties *Viorica* (V), *Legenda* (L) have been selected for research. The wines have been produced in 2017 at micro-winery of Enology department, Technical University of Moldova by using general technologies of white winemaking. The sulphur dioxide (SO_2) has been added in grape crusher (50 – 75 mg /kg). The grape must has been macerated 2 hours at 12 – 14°C. In the grape must during maceration have been added enzymes Ultrazym® 100G (Novozymes A/S, Denmark) (0,5 – 1 g/dl). From the grape variety Legenda the samples also have been taken directly from the grape press without maceration (L1), after 4 hours maceration (L2) and after 2 hours maceration (L3). For all wines the post-fermentation period lasted 40 days (14 – 16ºC). The wine samples have been filtered through the filter of 0,45 μ for spectrophotometric

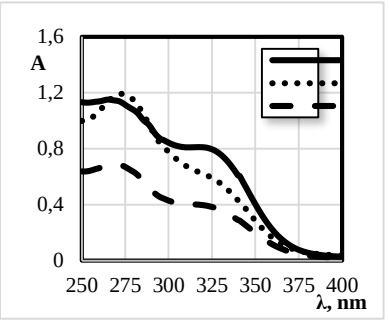
investigations (absorption spectra, total polyphenol index – IPT, phenolic compounds, the test of oxidation behavior – POM-test, antioxidant capacity of wines etc.). The spectrophotometric analyses have been done at single beam spectrophotometer PG T70 (PG Instruments, UK) and double beam spectrophotometer Specord 250 Plus (Analytik Jena, Germania). The comparative antioxidant capacity of wines has been determined using the method Crocin Bleaching Assay (CBA) [2, 3]. The absorbance capacity of Crocin has been measured at 443 nm. The generation of the radicals and its reaction with substrate have been performed in cuvettes held in thermostat at 40°C. Crocin has been extracted from commercial saffron (*Crocus Sativus L.*) (Aromatica SRL, Italia) and has been purified according to Ordoudi and Tsimidou [4]. The concentration of the extract has been determined by using spectrophotometric analysis. In reactant solutions with added wine is ensured the crocin concentration of 10^{-6} M. The concentration of total phenolic compounds, flavonoids and cinnamic compounds has been performed at the spectrophotometer according to Somers and Verette [5].

Results and discussion

The absorption spectra of the red wines in UV-vis region are measured at 260 – 280 nm. This value is based on the characteristic absorption of the benzene cycles of the majority of phenols at 280 nm. HCA (C6 – C3) are the major non-flavonoid phenolic compounds in white grape and wine. They have a maximum absorption at wavelength 300 – 350 nm. The visual analysis offers a first information about HCA content in the complex of total phenols. However, original spectra do not show the fine differences between studied samples, oxidized wine and unoxidized wine. This is possible if we study the second order derivative spectra. The minimum interdependences $d^2A/d\lambda^2$ show the exact positions of the obvious and latent maximum. The final spectra, the algebraic sum of individual spectra, can distinguish them and have no coincidence of maximum values with the values presented in the specialty literature. The second order derivative spectra are more sensitive at quantitative and quality changes of wines and allow to find out the differences by using spectrophotometry, a method more accesible than chromatography. The absorption spectra UV-vis of experimental wines *Legenda* without subsequent treatment (L1, L2, L3) and their second derivative spectra are shown in the figure 1. The second order derivative spectra in original forms are similar and highlight the essential differences in different spectral ranges.



a



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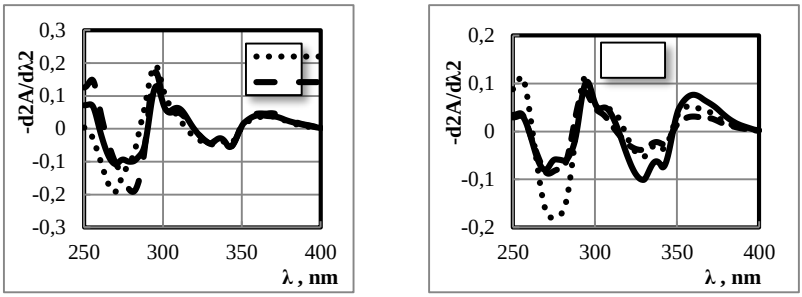


Fig. 1. *The absorption spectra of experimental wines Legenda (L1, L2 and L3 – a), Viorica, Chardonnay şi Sauvignon (V, Ch, S – b) and its second order derivative spectra (c and d).*

The major differences have been revealed at wavelength 260 – 280 nm, 300 – 315 nm and to a lesser extent at 330 – 345 nm. The sample with the less level of browning had a minimum of second derivative spectra at 269 nm and it had nearby the inflection point (281 nm), while the most oxidized wine (L2) had the minumum value at wavelength of inflection point L1.

The wine (L3) with intermediate browning have 2 minimum values in these positions (270 and 279 nm). This fact allow us to consider wavelength 269 – 270 nm suitable to maximum of unoxidized polyphenols, while the products of its oxidation correspond with wavelength 279 – 281 nm. The differences in the range 300 – 315 are less expressed where the second order derivative spectra have no extreme points. The more oxidized samples (L2 and L3) are characterized by minimum – maximum wavelength at 304 and 309 nm respectively. The samples L2 and L3 have more expressed minimum values than L1, because they have a higher content of oxidized polyphenols. These differences can be used for monitoring the oxidative processes in wines produced from *Legenda* grape variety.

The significant differences according to level of oxidation have been observed and in the visible region. These differences generally are quantitative, not qualitative. Legenda wines have no obvious or latent maximum values in the visible region. The absorbance at 420 nm for oxidized samples L2, L3 is 0,538 and 0,567 (l=1cm) and 0,164 for sample L1. *Viorica*, *Chardonnay* and *Sauvignon* wines in UV-vis region have absorption spectra with 2 distinct regions 250 – 300 nm and 300 – 350 nm. They are similar to *Legenda* wine spectra (Fig. 1). Absorption spectra show an increased HCA content in *Viorica* wine. The qualitative differences are more evident in second order derivative spectra. The same groups of minimum values in 300 – 350 region have been observed. In the interval between 250 – 300 nm, 2 minimum values 270 nm, 281 nm are evident for *Viorica* wine. In the case of Sauvignon wine prevails the first minimum value (272 nm), the second minimum is masked and is presented by an inflection point. At Chardonnay wine the both minimum have the very close values and its superposition gives a single band with λ_{min}=274 nm.

The concentration of total phenolic compounds – SFT (Galic Acid Equivalents, mg/l), phenolic cinnamic compounds – SFC (Caffeic Acid Equivalents, mg/l) and phenolic flavonoid compounds – SFF (Catechin Equivalents, mg/l) are presented in table.

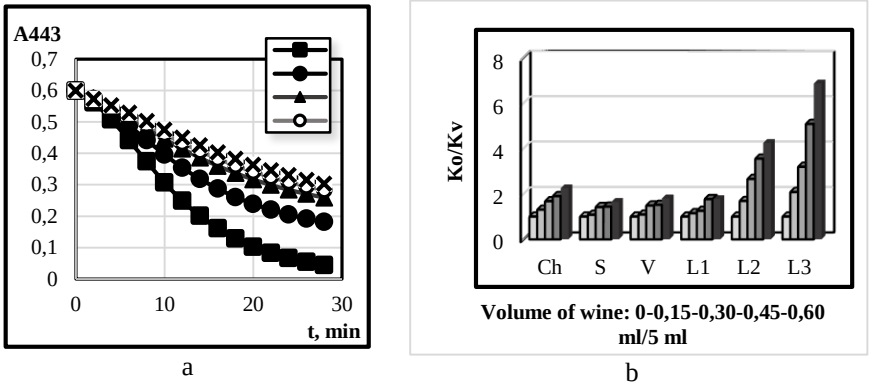
The maceration of L2 and L3 wines have been ensured the better extraction of HCA, total phenolic (SFT) and phenolic flavonoid compounds (SFF) than in L1 wine. The POM-test shows the increased oxidation behavior in L1 wine. This fact can be explained by the presence in the respective samples of unoxidized forms of HCA. The smallest value of POM-test have been identified in Chardonnay wine samples.

Table 1. *The concentrations of main phenolic compounds, the index of oxidation BEHAVIOR (POM-test) and the parameter of relative antioxidant capacity (K)*

Parameter	L1	L2	L3	V	Ch	S
SFT (Galic Acid Eq., mg/l)	145,1	269,4	236,1	199,3	220,4	68,8
SFC (Caffeic Acid Eq., mg/l)	29,6	56,2	51,7	67,0	48,1	25,7
SFF (Catechin Eq., mg/l)	206,2	377,0	319,1	160,1	298,7	43,6
IPT	8,95	12,98	11,94	10,76	11,47	6,59
POM-test (%)	81,8	74,2	42,1	24,0	10,1	67,7
K (Ko/Kv÷f(v wine))	1,34	5,46	9,14	1,.9	2,07	1,06
R^2	0,8964	0,9960	0,9759	0,9409	0,9793	0,9359

The value of POM-test for Sauvignon wine can be the consequence of decreased content of phenolic compounds extracted during processing. Our experiments shows that wine treatment with bentonite and PVPP can reduce efficiently the content of browning substances.

The antioxidant capacity of experimental wine samples have been determined by watching the competitive kinetics of Crocin color fading. The antioxidant capacity have been expressed by the interdependance between constant of color fading speed in absence of wine addition (Ko) and in the presence of wine addition in different volumes (v), in the reactant mixture (5 ml), in the spectrophotometer cuvettes (Kv). The monitoring of process has been done at 443 nm. In the figure 2 a is shown the kinetics curves of Crocin oxidation in the presence of different concentrations of *Chardonnay* wine.



a b

Fig. 2. *The kinetics of oxidative color fading of Crocin (CBA) in absence and presence of different concentrations of Chardonnay wine (a); dependence of comparative antioxidant capacity Ko/Kv on wine concentration in reactant solution (b).*

The interdependencies Ko/Kv for all studied wines show more moderate antioxidant capacity at Sauvignon, Viorica, L1 wines and unexpected expressed antioxidant capacity at L2 and L3 wines (Fig. 2 b). The close correlation between

antioxidant capacity and content of SFT, SFC and SFF have not been found, although the trend have been barely observed. The oxidation behavior of browning L2 and L3 wines can be explained due to high content of compounds mentioned before and possible antioxidant capacity of some products of browning, although have not been identified direct connection between the antioxidant capacity and browning degree of Maillard products in hydrophilic medium. The strict elucidation of these interdependencies requires the complex investigations for determine the influence of different wine antioxidant compounds, for reveal the possible effect of endogenous antioxidants of wine, the redox transformations that are catalized by transition metals, enzymes. The constants of linear dependencies Ko/Kv on the wine concentration in the reactant mixture have been determined with high values of correlation coefficient R^2 (Tab. 1).

Conclusions

The oxidation of hydroxycinnamic complex in experimental white wines Legenda, produced by using different technologies, have been studied. The oxidation of hydroxycinnamates were accompanied by quantitative spectral changes in visible light region and qualitative spectral changes in UV region. The POM-test offers the possibility to predict the risk of browning the white wine. There are no interdependence between the POM-test and total antioxidant capacity determined with Crocin.

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